(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 355 266 B1**

(12) # EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification: **09.06.93** (51) Int. Cl.5: **B01J 19/00**, C07K 1/04

(21) Application number: **89107707.5**

(22) Date of filing: **27.04.89**

(54) **Apparatus for performing repetitive chemical reactions.**

(30) Priority: **29.04.88 US 188453**

(43) Date of publication of application:
28.02.90 Bulletin 90/09

(45) Publication of the grant of the patent:
09.06.93 Bulletin 93/23

(84) Designated Contracting States:
DE FR GB

(56) References cited:
EP-A- 130 739
WO-A-82/03077
US-A- 3 188 181

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EP 0 355 266 B1

Description

Field of the Invention:

This invention relates to systems and procedures for chemical analysis, sequencing operations and synthesis. More particularly, the invention relates to an apparatus and process for the automated synthesizing of proteins, especially peptides.

Background of the Invention:

The accurate dissolution, dispensing and reaction of chemicals has numerous applications. These include analytical procedures such as, for example, the derivitization for HPLC determination of amino acid composition; sequencing operations such as in the Edman degradation procedure; and in synthesis of various substances, such as RNA, DNA, peptide and oligosaccharide assemblies.

Moreover, a growing number of research facilities, especially non-chemical laboratories, require synthetic peptides. In conventional practice, the production of synthetic peptides requires the work of chemists who are highly skilled in synthetic chemistry. This, and the necessity of exercising precise control of the chemicals to be added in a process, including quantity, sequence, timing, and the like, adds to the cost and time required to produce a given result, and may lead to inaccuracies.

Patents of interest which are exemplary of the state-of-the-art in this field are Verlander et al U.S. Patent No. 4,362,699, issued December 7, 1982, entitled "APPARATUS FOR HIGH PRESSURE PEPTIDE SYNTHESIS" and Bridgham et al U.S. Patent No. 4,668,476, issued May 26, 1987, entitled "AUTOMATED POLYPEPTIDE SYNTHESIS APPARATUS", the disclosures of which are incorporated herein by reference in their entirety.

Summary of the Invention:

Accordingly, it is an object of this invention to provide a simple and inexpensive automated system for the dissolution, dispensing and reaction of chemicals, especially for the synthesizing of peptides.

Another object of the invention is to provide a peptide synthesizer in which simplicity and low cost are achieved by utilizing fixed chemical protocols, pre-packaged Fmoc chemistry and a synthesis-dedicated computer.

A further object of the invention is to provide a novel apparatus and method for the storage and delivery of amino acids in the synthesizing of peptides and proteins.

Yet another object of the invention is to provide a system for the automated control of analytical procedures, sequencing operations and synthesis of chemicals.

Another object of the invention is to provide a novel means for the storage, dissolution and dispensing of a chemical, and its subsequent use as a pump in a reaction process involving the chemical.

An even further object of the invention is to provide a means for sequentially advancing containers of a chemical to a work station.

Still another object of the invention is to provide a unique, reusable reaction vessel having means permitting replacement of solid supports in the vessel.

The foregoing and other objects and advantages of the invention are achieved with an automated system including a synthesis apparatus and a computer-operated control. The apparatus includes a novel fluid transport system that incorporates a syringe-type amino acid dissolution and delivery system with a flow-through reusable reactor. The computer control utilizes a pull-down menu and requires a minimum of key-strokes, thus making possible operation by most, if not all, laboratory personnel.

For synthesis of peptides, the invention makes particular use of the fact that mixtures of solid Fmoc-protected amino acids and BOP (benzotriazolyloxytris-(dimethylamino)phosphonium hexafluorophosphate, optionally with equimolar amount of HOBt (hydroxybenzotriazole), are stable when stored under dry conditions. When these are dissolved in activator (N-methylmorpholine in DMF), rapid formation of a highly active (greater than symmetric anhydrides) and long lasting intermediate occurs. The chemistry protocol follows the basic Fmoc deblock-couple cycle, but the long lived intermediate allows the coupling time for difficult sequences to be extended rather than resorting to the "double coupling" schemes used in the prior art.

The mixtures of amino acid, BOP and HOBt are provided in disposable syringe-type cartridges. The cartridges are loaded in a rotating carousel for sequential access under control of the automated system. The solid supports are loaded into the reusable reaction vessel, and prepared reagent solutions are stored and dispensed from a plurality of reservoirs contained within the apparatus. Bulk solvents and waste solutions are contained in suitable external reservoirs.

After entering a desired sequence at the computer work station with appropriately written software programs, the user can choose to have the apparatus calculate reagent requirements for the synthesis. These calculations can then be used as a guide in loading the reagents in the synthesizer.

The cartridge in which the amino acid powder is stored functions not only to preferably store the amino acid in a dry, hermetically sealed environment, but after connection to the fluid transport system, also functions as a syringe pump to draw solvents and reagents into the cartridge for dissolution and reaction of the contents when the syringe plunger is raised, and for then expelling the dissolved and reacted materials to a further reactor or reactors or analysis unit or units by depressing the plunger of the syringe. Following initial dispensation, the solution may be drawn back into the cartridge for subsequent delivery to further reactors or analysis units. In a particular application, e.g., peptide synthesis, the reaction solution can advantageously be reciprocated backwards and forwards between the reactor(s) and the cartridge, providing suspension and re-suspension of the synthesis support material(s), thereby assuring uniform and near quantitative reaction. The reciprocation further aids in dissolution of poorly soluble materials.

After dissolution and dispensation of its contents, the cartridge serves as a syringe pump for the metering, dispensation and mixing of other reagents and solvents accessible from the fluid system. Because of their accuracy and variable dispensation rates, syringe pumps are attractive for administering fluid dispensation in synthesis, sequencing and analytical instrumentation. However, they have not been widely used because of reliability problems. The disposable syringe of the present invention solves this problem since it can be, and preferably is, discarded after each cycle of operation.

The carousel in which the cartridges are carried and supported for sequential access has a plurality of radial slots in its periphery for receiving the cartridges and includes a clamping structure for securely clamping the cartridges in place. The carousel is indexed through predetermined arcs of movement to bring the cartridges into operative position for dissolution, reaction and dispensation of the contents of the cartridges. Indexing of the carousel is controlled by a motor operated in response to strategically placed sensors and the computer control system.

A plunger gripping and actuating device is positioned above the carousel in position to intercept and engage a flange on the cartridge plunger as the carousel is indexed, and thereafter to move the plunger up and down to draw a solvent and/or reagent into the cartridge to dissolve and dispense the contents into the fluid system for reaction with selected reagents contained in suitable reservoirs. Under control of the computer system, the plunger gripping and actuating device reciprocates the plunger to cause the cartridge and plunger to function as a syringe pump for mixing, etc., of the contents of the cartridge with other reagents, etc, contained in the fluid system.

The fluid system includes a plurality of valves which are operated in a predetermined sequence to introduce different materials, and/or to cause flow of the material(s) to and from different parts of the system.

A novel reactor column for containing the solid supports used in peptide synthesis, for example, has a snap-together body for access to the solid supports.

Brief Description of the Drawings:

The foregoing and other objects and advantages of the invention will become apparent from the following detailed description when considered with the accompanying drawings, wherein like reference characters designate like parts throughout the several views, and wherein:

Fig. 1 is a schematic diagram of the system used in the invention;

Fig. 2 is an enlarged, longitudinal sectional view of the unique cartridge used in the system of the invention;

Fig. 3 is an enlarged, fragmentary sectional view of a portion of the cartridge and carousel, showing the manner in which the cartridge is gripped and held by the plates of the carousel;

Fig. 4 is bottom plan view of the carousel, with a portion thereof broken away, showing the slotted configuration and the relationship of the flange on the cartridge to the slotted carousel plates;

Fig. 5 is an enlarged, fragmentary top view of a portion of the plunger gripper, showing its relationship to a cartridge as the cartridge advances toward operative relationship with the gripper;

Fig. 6 is a side view in elevation, with portions broken away, of the carousel, gripper and fluid system connection used in the system of the invention.

Detailed Description of the Preferred Embodiments:

With more particular reference to the drawings, the system of the invention is indicated generally at 10 in Fig. 1. As defined hereinafter, the system is intended for synthesis of peptides, but, as noted earlier, it could also be used for other processes. The system includes a carousel 11 in which the cartridges 12 are supported; a linearly actuated gripper mechanism 13 for gripping and reciprocating the plunger 14 of a cartridge; a fluid transport system 15, including a fluid connector 16 and reactor column 17; and an electronic control including board 18.

The syringe-type cartridge 12 comprises a modified disposable syringe designed for automation. The cartridge is initially used for storage of the solid amino acid mixtures "M", and includes an elongate cylindrical body 20 having an open upper end 21 and a reduced diameter lower end 22 having a tapered bore 23 therein defining a friction fit female connection. The cartridge volume should preferably be about 5 ml. A diametrically enlarged gripping flange 24 is formed on the outside of the body near the lower end thereof. As seen best in Fig. 2, a foraminous block or frit 25 is secured within the body in the area of the flange 24 and defines a mechanical barrier against leakage of the solid reagents. The frit preferably has a filtration ability of about 125 microns for the materials described herein.

The plunger 14 is slidably sealed in the cylindrical housing or body, whereby the solid amino acid mixture "M" is confined within the cartridge body between the frit 25 and the plunger 14. The plunger has an enlarged flange 27 on its outer, free end, for a purpose to be later described, and as assembled and ready for use, is positioned about one-half the distance into the cartridge. Pumping action of the plunger in the cartridge is capable of drawing 2.5 ml of DMF (dimethyl formamide) and amino acid back through the reaction column 17 as well as pushing it forward through the column and is capable of moving a volume of 5 ml in either direction during a full stroke.

A hermetic seal 28 is secured across the lower end of the cartridge to prevent atmospheric contamination of the material M. A recessed area 29 is formed in the cartridge behind the seal to provide an area for receipt of the seal when it is ruptured by the male connector 50, to prevent interference with the liquid seal effected by the connector 50.

The cartridge and plunger are made from a suitable disposable material determined to be inert to the chemicals associated with peptide synthesis, such as Teflon, and, in a preferred embodiment, the cartridge body 20 will be injection molded from low-density polyethylene (LDPE) while the plunger 14 will be injection molded from polypropylene. The frit 25 is also manufactured from polypropylene and may be purchased from Porex Technologies, Stock No. X-5616. The seal 28 comprises a thermoplastic coated foil membrane and may be purchased from 3M and cut to size. The seal is applied to the cartridge body by induction heating using commercially available equipment (not shown or described).

The carousel 11 is removably supported on a bearing block 30 and is driven via belt 31 and pulley 32 by a motor 33. The carousel comprises a top plate 34 having a plurality of radial slots 35 in its peripheral edge, and a cartridge spring plate 36 engaged beneath the top plate. In use, the flange 24 on the cartridge 12 is engaged between the top plate 34 and spring plate 36, with the cartridge body extending through the slot 35 so that the lower end thereof with the female connector 23 is positioned below the spring plate and the upper end with the plunger 14 is positioned above the top plate (see Figs. 1 and 6).

Suitable indicia 37 is provided on the bottom surface of the spring plate in a position to be detected by a position sensor S_1, which controls operation of the motor 33 and defines a "parked" or home position for the carousel. Another sensor S_2 detects the position of the most recent "spent" cartridge 12A.

In a preferred embodiment, a plurality of slots 35, preferably 40, will be provided in the carousel for holding forty cartridges. The cartridges in the embodiment described herein are hand loaded into the slots in a predetermined order depending upon the intended use for the apparatus. After the cartridges have been loaded into the carousel, the carousel is placed in the apparatus and "home" position determined by the location of the indicia 37 and sensor S_1. Alternatively, the carousel may be loaded when the carousel is in place on the bearing block 30.

The gripper mechanism 13 comprises a linear actuator 40 connected to an arm 41 midway between the ends of the arm. The arm has a slide 42 on one end riding on a track 43, and a plunger gripping slot 44 on its other end. Thus, operation of the actuator 40 causes the arm 41 to move up and down in a straight line or vertical path relative to the carousel and a cartridge 12 supported therein.

Upon initiation of a cycle of operation (via appropriate command given through the computer and the board 18) and loading of the carousel as described above, the gripper arm 41 is lowered in front of the next succeeding cartridge plunger flange 27 until the flange is directly in line with the gripper slot 44 on the arm 41. Optical or other suitable sensors detect and insure the precise location. The carousel is then advanced to engage the plunger flange 27 with the gripper slot 44.

The fluid connector 16 is positioned beneath the carousel in position to be in alignment with the lower end of a cartridge when the cartridge is positioned to engage the flange of its plunger with the gripper slot 44, as described above, and comprises a male connector 50 (see Fig. 2) having a tapered end 51 for piercing the foil seal 28 on the cartridge lower end and making a frictional engagement in the female recess. The male connector is mounted through a spring-loaded connection on a reciprocable member 52 operated by pneumatic motor 53 via valves V_1 and V_2 from unregulated supply line 54. The male connector 50 is con-

nected with the fluid transport system 15 via a fluid line 55 extending between the connector and a valve V_3. A fluid sensor S_F is associated with line 55 to detect the presence of fluid in the line. Thus, when the cartridge has been advanced and its plunger flange engaged with the gripper slot 44, the male connector is actuated to pierce the seal 28 and establish fluid communication between the contents of the cartridge and the fluid system.

After connection of the cartridge to the fluid transport system, the gripper mechanism is raised, thereby raising the plunger and drawing solvent and reagent into the cartridge to be admixed with the amino acid mixture. Several different reservoirs R_1, R_2, R_3, R_4, R_5 and R_6 are provided, for containing one or more reagents. Each reservoir has a three-port, two-way valve connected with it, as at VR_1, VR_2, VR_3, VR_4 and VR_5, respectively. In addition, one of the reservoirs, R_6, comprises an external, four liter bottle and there are three three-port, two-way valves VR_6A, VR_6B and 74 connected with it. As seen in Fig. 1, the valves VR_1, VR_2, VR_3, VR_4 and VR_5 are arranged in two banks B_1 and B_2 associated with the reservoirs R_1 and R_2, and with R_3, R_4 and R_5, respectively. External reservoir R_6 is connected via its valves to each of the banks.

A further three-port, two-way valve 60 is connected in a fluid conduit 61 extending between the two banks of valves, and two two-port valves 62 and 63 are interposed in this conduit between the valve 60 and each bank of valves.

A waste conduit 64 leads from the valve 60 to a suitable waste disposal site, and a two-port valve 65 is interposed in this conduit. A fluid sensor S_F is associated with this waste conduit to detect the presence of fluid in the conduit.

Fluid conduit 61 and bank B_2 are connected to different ports of a three-port, two-way valve 70, which is, in turn, connected to the lower end of reactor column 17. A fluid sensor S_F associated with the conduit 71 leading from the valve 70 to the reactor column 17.

The upper end of the reactor column is connected via conduit 72 to series-connected three-port, two-way valves 73 and 74 and two-port valve 75. An outlet conduit 76 leads from valve 73 to waste, and a conduit 77 connects valve 74 with the reservoir R_6. Valve 75 is connected via conduit 78 with a first manifold 79 for distributing pneumatic pressure to the system.

Manifold 79 is also connected via conduit 80 and two-port valve 81 with the first bank B_1 of valves, and via conduit 82 and two-way, two-position valve 83 to the bank B_2 of valves.

A second manifold 90 for distributing pneumatic pressure is connected via conduits 91, 92, 93, 94, 95 and 96 wit the respective reservoirs for pressurizing the contents of the reservoirs to assist in the movement of fluids through the system. Venting of all reservoirs is provided by a manual toggle 103. The line 93 to the piperidine deblock reservoir R3 is also equipped with a non-return check valve 106 to prevent contamination of other solvents and reagents by piperidine vapor.

The first manifold 79 is provided with regulated pressurized gas via conduit 100 and pressure regulator 101, while the second manifold 90 is provided with regulated pressurized gas via conduit 102, regulator 101 and three-port, three-way valve 103 operated either manually or by pressure switch 104.

Gas to the system is through flow meter 105 and, in a preferred embodiment, is at 30 psi, regulated to about 6 psi. Additionally, the gas is inert with respect to the various chemicals used in the process.

The valves are solenoid controlled, and with the fluid sensors and motor controls MC_1 and MC_2 are connected with controller 110, which is responsive to commands from the board 18 and computer (not shown) A communication port 111 is provided on the board for connection to the computer, and a suitable power supply 112 is also connected to the board.

The fluid conduits, valve components, manifolds, reservoirs and other components coming into contact with the fluids being handled by the system are made from a material inert to the fluids, such as Teflon, polypropylene, polyethylene and stainless steel. The gas used to provide pneumatic pressure in the system will be an inert gas, such as nitrogen, argon or helium. Selection of the gas will be determined by its mixing characteristics within the reactor column. Further, the cartridges may be provided with a bar code and a suitable reader 113 positioned to sense the bar code for confirmation of proper cartridge position and sequence.

The reagent reservoirs, valves and electronics are supported in a sheet metal housing. The electronics and pneumatics may be housed in a tray assembly that can be removed from the rear of the housing; and the carousel, gripper assembly and fluid connection structure comprise a single, replaceable assembly. A large door in the front of the housing permits access to the five fixed position reagent bottles. Most of the valves and the pneumatics are also accessible through doors in the rear of the housing.

Alongside the housing are three one gallon bottles: one for DMF and the other two for waste. Special vapor traps containing Dowex 50W-X8 ion exchange resin (sulfonic acid) allow the waste bottles to be vented directly into the laboratory.

In a typical setup for peptide synthesis, each cartridge will contain 0.5mmol of Fmoc amino acid,

HOBt and BOP. The plunger will be inserted halfway into the cartridge, defining a volume of 2.5ml in which the dry mixture is stored. The three component amino acid mixture is activated by withdrawing the plunger, drawing activator into the cartridge. The amino acid is dissolved and activated simultaneously. It is then expelled from the syringe on the downward stroke of the plunger and directed into the reactor column. The porous frit in the bottom of the cartridge acts to prevent insoluble residues or reaction by-products from entering reaction and valving systems. By reciprocating the plunger, the amino acid solution, as well as up to 5ml of other synthesis reagents may be continuously moved through the reactor column. The reciprocation further aids in the mechanical dissolution of poorly soluble materials.

The cartridges are designed to last for 200 pumping cycles. Each spent cartridge is replaced at the end of the coupling cycle by a fresh cartridge containing the next amino acid in the sequence.

To disengage the cartridge plunger, all fluid is first expelled from the cartridge. The fluid supply lines are then disconnected and the carousel is advanced until the gripper is clear of the plunger. The gripper is then fully raised and the carousel advanced to bring the next cartridge into operative position for engagement with the gripper, and the operation as described above repeated.

In a typical operation involving peptide synthesis, there are only two distinct procedures within the Fmoc coupling cycle: deblock and couple, separated by an efficient DMF wash, see, e.g., copending Hudson application Serial No. 044,185, filed April 30, 1987, and Melenhofer, U.S. Patent No. 4,108,846, issued August 22, 1978, and entitled "SOLID PHASE SYNTHESIS WITH BASE N ALPHA-PROTECTING GROUP CLEAVAGE", both of which are incorporated herein by reference in their entirety. Synthesis is carried out on either Pepsyn K or polystyrene solid supports. Initial deblock of the resin is achieved with 30% piperidine in DMF for 10 minutes, followed by 6-10 washes with DMF. The cycle is then begun by activating and coupling the amino acid as follows: 0.5mmol of the Fmoc-amino acid, BOP and HOBt mixture is dissolved in 2.5ml of activator and coupled to 0.1mmol of support. Reaction times of 20 and 40 minutes are used, with continual reciprocation of the reaction mixture achieving uniform and efficient reaction. Subsequent to coupling, washing of the reactor is performed with DMF, and the cartridge is deblocked with 30% piperidine in DMF for 10 minutes, followed by 6-10 washes with DMF to end the cycle. The cartridge is then disposed of and a new cartridge positioned to repeat the cycle.

The same apparatus can be used in DNA synthesis, with 50-100 micromoles of support. Amidite derivatives are placed in the cartridges and then dissolved and activated by the addition of tetrazole in acetonitrile. Since the amidites are stored as solids, the problem of decomposition is obviated. Large excesses, e.g., 20-50 fold, are currently used in DNA synthesizers. The efficient mixing, washing and lack of decomposition provided by the invention permits operation with only a five fold excess. Consequently, large amounts of DNA can be prepared rapidly and economically.

Typical control commands can be employed and other functions can be added or substituted in the foregoing system which alterations should be within the purview of one skilled in this art. In operation, typically, the following steps will be employed.

Solid phase peptide synthesis with the Excell involves the following steps:

1. Insert sequence commands to computer control.
2. Place support material in the column reactor.
3. Load cartridges and verify correctness with bar-code reader.
4. Load reagents and solvents.
5. Start up synthesis consisting of priming lines and washing reactor column.
6. Fmoc-removal. A blank cartridge measures and mixes piperidine with DMF. The removal reagent is reciprocated between the reactor and the syringe. After 3 minutes, this reagent is replaced with freshly diluted solution and removal contained for another 7 minutes.
7. Syringe is washed and dispensed with.
8. Column is washed (by bi-directional flow) to remove all piperidine.
9. All lines blown dry with argon.
10. Activator solution admitted to next cartridge containing the Fmoc-amino acid, BOP and HOBt. This effects complete dissolution, rapidly converts the amino acid into a form which will couple, and is transferred to the reactor and reciprocated to achieve uniform and complete reaction.
11. After coupling, excess amino acids are washed out of the system.

This completes one cycle of addition, this process is continued until the desired sequence is assembled, then the support is removed and the peptide obtained by mild acid cleavage.

The following is a specific description of operation of the embodiment described for peptide synthesis. The processes described, including priming, washing, deblocking, purging and coupling are general for any synthesis application. Flow and control for other applications may be varied to suit a specific synthesis operation.

1. User sequence selection or entry.
2. Set up. The proper reagents and solvents are placed in the reservoirs according to amounts calculated by the controller. The amino acid cartridges are then placed in the carousel in the correct sequent to be assembled. A blank cartridge is placed in the first position. Loading can be prompted by a display on the computer controller. The carousel next rotates the cartridges past the bar-code reader before any operation of the machine to VERIFY that loading has been performed correctly. Unnatural acids, D-amino acids, and user specific amino acids can be accommodated by the bar-code system used. Operation in the absence of verification is also possible.
3. Start up. Upon commencing operation, all reagent and solvent lines are primed. This involves opening the valve and waste (e.g., Act 1 is primed by operating VR_7, VR_1, 65, 60, and 62 for 4 seconds). All priming routines by-pass the column.
4. Valve train wash. DMF is washed through the activator and reagent valve trains to remove contamination (VR_7, VR_6A, 65, 60, 62) followed by (VR_8, VR_6/B, 63, 65 and 60).
5. The column and its contents, the synthesis support, are next thoroughly washed. This process consist of: i) upward washing (opening valves 73, VR_8, VR_6/B, 70); ii) a pause (8 seconds); iii) downward washing through column 17 (74, 70, 63, 65, 60); and iv) emptying of column 17 (75, 70, 63, 65, 60) by argon. Steps i) to iv) are then repeated once.

iv) The lines and column are then purged of all fluid with argon.

6. Deblock reagent dilution. The gripper 13 engages the first EMPTY cartridge, completely depressed the plunger, and then partially raises the plunger to admit 3 parts of piperidine (VR_8, VR_3, VR_9, 63, 62, V_3), the plunger is further raised to admit 7 parts of DMF (VR_8, VR_6/B, 63, 62, V_3).
7. Column deblocking, first treatment. The plunger is depressed delivering entire contents to column reactor (73, 70, 63, 62, V_3). After a pause (8 seconds), the deblocking mixture is drawn back into the syringe, then re-expelled. This process is continued for 3 minutes.
8. Column deblocking, second treatment. the contents of the syringe are dispelled to waste (plunger down, V_3, 62, 60, 65). The processes described in 6 and 7 above are then repeated to dilute further piperidine to 30% and perform deblocking for a second period of seven minutes.
9. Syringe emptying and line purging with argon to displace most piperidine. Syringe then filled with DMF (VR_7, VR_6A, V_3) and left.
10. Column is washed 6 times with DMF as described in Section 5, i)-iv).
11. Syringe emptied to waste. Then filled with DMF through the column (74, 70, 63, 62, V_3) and emptied to waste (V_3, 62, 60, 65). Repeated 4 times.
12. All lines and column are purged of fluid.
13. Gripper 13 disengages from spent cartridge. New cartridge is placed in position by advancing carousel and gripping plunger.
14. Plunger is depressed (V_3, 62, 60, 65). Then withdrawn to admit 2.5 ml activator (0.3M N-methylmorpholine in DMF) (VR_1, VR_7, V_3).
15. Coupling is performed by expelling activated amino acid to column (73, 70, 63, 62, V_3), then reciprocating fluid to ensure mixing, uniform reaction and complete amino acid dissolution. The withdrawal step involves activation of valves 75, 70, 63, 62 and V_3.
16. After adopted coupling time, the spent amino acid solution is displaced to waste. The cartridge is filled with DMF (VR_7, VR_6A, V_3) and left whilst the column is washed as in 5, i)-iv). Cartridge washing, as in 11, is then performed.
17. Fluid purging from system with argon.
18. Steps 6 to 17 are then repeated to assemble the desired sequence.
19. Final Fmoc group may be left on or removed.
20. Synthesis ends with methanol and methylene chloride washed and nitrogen purge through column.
21. Support removed from column and cleaved.

The foregoing system, system components, controls and method of operation are exemplary only and different synthesis, and equivalent apparatus, may be substituted for that disclosed, where appropriate, and still achieve the overall improvements taught herein. The scope of the invention is only limited by the claims and the applicable prior art.

Claims

1. An installation for automated chemical processing, comprising:
   a plurality of chemical storage cartridges (12), at least one cartridge (12) for storing a first chemical;
   means (13) for choosing between any one of said cartridges (12) and holding said chosen cartridge (12) and positioning said cartridge (12) a priori at a fixed location within said system;
   a reservoir (Rn) spaced from said location for containing a second chemical to be reacted with said first chemical in said chosen car-

tridge (12) to produce a third chemical;
means (16) at said location for fluid coupling to said chosen cartridge (12) to establish fluid communication with the contents thereof;
a fluid conduit (15) connected to said fluid coupling means (16) and said reservoir (Rn) to dissolve and conduct said first chemical from said chosen cartridge (12) to said reservoir (Rn) to react with said second chemical;
valve means (Vn) operably connected with said fluid conduit (15) for controlling flow through said conduit;
said cartridges (12) including pump means (14) operable when said chosen cartridge (12) is positioned at said location for changing the internal volume thereof so as to cause bi-directional flow of said first chemical through said fluid coupling means (16), alternatively into and out of said reservoir (Rn) and said chosen cartridge (12) to promote mixing and reacting with said second chemical in said reservoir (Rn) to complete the formation of said third chemical;
automatically operated control means (18) connected with said pump means (14) and said valve means (Vn) to obtain a predetermined sequence of operation of said pump means (14) and said valve means (Vn) for controlling the dispensing of said first and second chemicals and the further processing of said third chemical.

2. The installation as claimed in Claim 1 wherein:
a source of pneumatic pressure is connected with said reservoir (Rn) to pressurize the contents thereof to assist in flow of said first chemical into and out of said reservoir (Rn).

3. The installation as claimed in Claim 2 wherein:
said system comprises a protein synthesis apparatus, and said reservoir (Rn) includes a reactor column (17) connected in series with said fluid conduit (15) for receiving said first chemical from said chosen cartridge (12).

4. The installation as claimed in Claim 3 wherein:
said automatically operated control means includes a computer (18) programmed to operate said valve means (Vn) and said pump means (14) in said predetermined sequence.

5. The installation as claimed in any one of Claims 1-4 wherein:
said pump means comprises a plunger (14) slidably sealed in said cartridges (12) and a reciprocating arm (40) connected with said plunger (14) to reciprocate said plunger (14) when said chosen cartridge (12) is at said location to alternately draw material into said chosen cartridge (12) and then expel the material from said chosen cartridge (12).

6. The installation as claimed in Claim 5 wherein:
said cartridge choosing means (13) comprises a rotating carousel (11) having means (35, 36) for holding and supporting said cartridges (12) thereon; and
said control means (18) is connected to operate said carousel (11) in timed sequence with operation of said valve means (Vn) and said pump means (14).

7. The installation as claimed in Claim 6 wherein:
said fluid coupling means (16) includes means (16) movable into and out of contact with said chosen cartridge (12) at said location; and
said control means (18) is connected to operate said fluid coupling means (16) in timed sequence with operation of said carousel (11), said pump means (14) and said valve means (Vn).

8. The installation as claimed in Claim 7 wherein:
sensors are positioned adjacent said carousel (11), said pump means (14) and said fluid coupling means (16) to detect the position and status thereof and in response thereto to send a signal to said control means (18) to insure operation of said carousel (11), pump means (14) and fluid coupling means (16) in proper timed sequence.

9. The installation as claimed in Claim 8 wherein:
said carousel (11) comprises a disc-shaped member (34) having a plurality of slots (35) in a peripheral edge thereof, said cartridges (12) being supported in each one of said slots (35).

10. The installation as claimed in Claim 3 wherein:
said first chemical in said cartridges (12) comprises a mixture of solid amino acid, hydroxybenzotriazole and benzotriazolyloxytris-(dimethylamino)phosphonium hexafluorophosphate;
said second chemical in said reservoir (Rn) comprising N-methylmorpholine in dimethylformamide for dissolution and activation in said first chemicals in said chosen cartridge (12) to form said third chemical; and
a polystyrene solid support included in said reactor column (17) for coupling with said activated third chemical from said chosen cartridge (12).

11. The installation as claimed in Claim 5 wherein:
said cartridges (12) include a diametrically en-

larged flange (24) formed on the outside of the body (20) thereof between the ends (21, 22) thereof for attachment of said cartridges (12) to a support apparaturs.

12. The installation as claimed in Claim 11 wherein:
a porous member (25) is secured in said body (20) adjacent to and covering the lower end (22) thereof to define a mechanical barrier to leakage of chemical from the interior of said cartridges (12).

13. The installation as claimed in Claim 6 wherein:
said rotatable carousel (11) for holding and advancing said cartridges (12) having an exterior flange (24) thereon, said carousel (11) further comprising:
a top plate (34) having a peripheral edge with a plurality of radially extending slots (35) therein; and
a bottom plate (36) secured beneath the top plate (34) to clamp between the top and bottom plates (34, 36) the flange (24) of one of said cartridges (12) received in a slot (35) in the top plate (34), whereby said one cartridge (12) is supported in an upright position by said carousel (11).

14. The installation as claimed in Claim 13 wherein:
said bottom plate comprises a spring plate (36) resiliently and yieldably biased against the underside of said top plate (34).

15. The installation as claimed in Claim 10 wherein:
said reactor column (17) for holding said solid supports comprises a molded body of material inert to chemicals being handled in said column (17), said body including an elongate inlet portion having opposite open ends, and an elongate outlet portion having opposite open ends, said inlet and outlet portions being releasably snap-fitted together at one of their ends in coaxial relationship with one another, a block of porous material secured in the other end of each of the inlet and outlet portions of provide a mechanical barrier to leakage of solid material from the column (17), and means on each of the other ends of the inlet and outlet portions for connection of said column (17) to a fluid delivery system.

Patentansprüche

1. Anlage für automatisierte chemische Verarbeitung oder Behandlung, umfassend:

eine Anzahl von Chemikalienspeicher-Patronen (12) mit mindestens einer Patrone (12) für die Speicherung oder Aufbewahrung einer ersten Chemikalie,

eine Einrichtung (13) zum Wählen einer gegebenen der Patronen (12) und zum Halten der gewählten Patrone (12) sowie zum Positionieren der Patrone (12) a priori an einer festen Stelle im System,

einen von der genannten Stelle beabstandeten Vorratsbehälter (Rn) zur Aufnahme einer zweiten Chemikalie, die mit der ersten Chemikalie in der gewählten Patrone (12) zwecks Erzeugung einer dritten Chemikalie umzusetzen ist,

eine an der genannten Stelle befindliche Einrichtung (16) für Fluidanschluß an die gewählte Patrone (12) zwecks Herstellung einer Fluidverbindung mit ihrem Inhalt,

eine an die Fluidanschlußeinrichtung (16) und den Vorratsbehälter (Rn) angeschlossene Fluidleitung (15) zum Auflösen und Führen der ersten Chemikalie aus der gewählten Patrone (12) zum Vorratsbehälter (Rn), um sie mit der zweiten Chemikalie umzusetzen,

eine betrieblich oder wirkungsmäßig mit der Fluidleitung (15) verbundene Ventileinrichtung (Vn) zum Steuern der Strömung durch die Leitung,

wobei die Patronen (12) eine Pumpeneinheit (14), die dann betätigbar ist, wenn die gewählte Patrone (12) an der genannten Stelle positioniert ist, um deren Innenvolumen zu ändern und damit eine bidirektionale Strömung der ersten Chemikalie durch die Fluidanschlußeinrichtung (16), abwechselnd in den und aus dem Vorratsbehälter (Rn) und die gewählte Patrone (12) herbeizuführen zwecks Begünstigung des Vermischens und Umsetzens mit der zweiten Chemikalie im Vorratsbehälter (Rn) für die Vervollständigung der Bildung der dritten Chemikalie, (und) eine automatisch betätigte, mit der Pumpeneinheit (14) und der Ventileinrichtung (Vn) verbundene Steuereinheit (18) zum Erreichen einer vorbestimmten Betriebssequenz der Pumpeneinheit (14) und der Ventileinrichtung (Vn) zwecks Steuerung der Abgabe der ersten und zweiten Chemikalien sowie der Weiterverarbeitung der dritten Chemikalie aufweisen.

2. Anlage nach Anspruch 1, wobei:
eine Pneumatikdruckquelle mit dem Vorratsbehälter (Rn) verbunden ist, um dessen Inhalt unter Druck zu setzen und damit die Strömung der ersten Chemikalie in den und aus dem Vorratsbehälter (Rn) zu begünstigen.

3. Anlage nach Anspruch 2, wobei:
das System eine Proteinsynthesevorrichtung umfaßt und der Vorratsbehälter (Rn) eine mit der Fluidleitung (15) in Reihe geschaltete Reaktorsäule (17) zum Aufnehmen der ersten Chemikalie aus der gewählten Patrone (12) aufweist.

4. Anlage nach Anspruch 3, wobei:
die automatisch betätigte Steuereinheit einen Rechner (18) aufweist, der für die Betätigung der Ventileinrichtung (Vn) und der Pumpeneinheit (14) in der vorbestimmten Sequenz programmiert ist.

5. Anlage nach einem der Ansprüche 1 bis 4, wobei:
die Pumpeneinheit einen unter Abdichtung in den Patronen (12) verschiebbaren Kolben (14) und einen mit dem Kolben (14) vorbundenen, hin- und herbewegbaren Arm oder Pendelarm (40) zum Hin- und Herbewegen des Kolbens (14), wenn sich die gewählte Patrone (12) an der genannten Stelle befindet, umfaßt, um abwechselnd Stoff in die gewählte Patrone (12) anzusaugen und dann den Stoff aus der gewählten Patrone (12) auszutreiben.

6. Anlage nach Anspruch 5, wobei:
die Patronenwähleinrichtung (13) einen rotierenden Drehtisch (11) mit Einrichtungen (35, 36) zum Halten und Tragen der Patronen (12) an ihm aufweist und
die Steuereinheit (18) angeschlossen oder geschaltet ist zum Betätigen des Drehtisches (11) in zeitlicher Folge mit der Betätigung der Ventileinrichtung (Vn) und der Pumpeneinheit (14).

7. Anlage nach Anspruch 6, wobei:
die Fluidanschlußeinrichtung (16) ein in und außer Berührung mit der gewählten, an der genannten Stelle befindlichen Patrone (12) bewegbares Mittel (16) aufweist und
die Steuereinheit (18) angeschlossen oder geschaltet ist zum Betätigen der Fluidanschlußeinrichtung (16) in zeitlicher Folge mit der Betätigung von Drehtisch (11), Pumpeneinheit (14) und Ventileinrichtung (Vn).

8. Anlage nach Anspruch 7, wobei:
neben dem Drehtisch (11), der Pumpeneinheit (14) und der Fluidanschlußeinrichtung (16) Sensoren angeordnet sind, um deren Stellung und Status zu bestimmen und in Abhängigkeit davon ein Signal zur Steuereinheit (18) zu liefern zwecks Sicherstellung der Betätigung von Drehtisch (11), Pumpeneinheit (14) und Fluidanschlußeinrichtung (16) in einer einwandfreien zeitlichen Folge.

9. Anlage nach Anspruch 8, wobei:
der Drehtisch (11) ein scheibenförmiges Element (34) mit einer Vielzahl von in einem Umfangsrand desselben vorgesehenen Schlitzen (35) aufweist und die Patronen (12) jeweils in einem der Schlitze (35) gehaltert sind.

10. Anlage nach Anspruch 3, wobei:
die erste Chemikalie in den Patronen (12) ein Gemisch aus fester Aminosäure, Hydroxybenzotriazol und Benzotriazolyloxytris(dimethylamino)phosphoniumhexafluorphosphat ist,
die zweite Chemikalie im Vorratsbehälter (Rn) aus N-Methylmorpholin in Dimethylformamid zum Auflösen und Aktivieren der ersten Chemikalie(n) in der gewählten Patrone (12) zwecks Bildung der dritten Chemikalie besteht und
in der Reaktorsäule (17) ein fester Polystyrolträger für Kupplung mit der aktivierten dritten Chemikalie aus der gewählten Patrone (12) enthalten ist.

11. Anlage nach Anspruch 5, wobei:
die Patronen (12) an der Außenseite ihres Körpers (20) zwischen ihren Enden (21, 22) einen diametral erweiterten Flansch (24) für Anbringung der Patronen (12) an einer Halterungs- oder Tragvorrichtung aufweisen.

12. Anlage nach Anspruch 11, wobei:
im Körper (20) im Bereich seines unteren Endes (22), und dieses bedeckend, ein poröses Element (25) zur Festlegung einer mechanischen Barriere gegen ein Herausdringen von Chemikalie aus dem Inneren der Patronen (12) festgelegt ist.

13. Anlage nach Anspruch 6, wobei:
der (rotierende) Drehtisch (11) zum Halten und Vorwärtsbewegen oder Weiterschalten der einen äußeren Flansch (24) aufweisenden Patronen (12) ferner umfaßt:
eine obere Platte (34) mit einem Umfangsrand, in welchem zahlreiche radial verlaufende Schlitze (35) vorgesehen sind, und
eine unterhalb der oberen Platte (34) befestigte untere Platte (36) zum Verspannen des Flansches (24) einer der Patronen (12), die von einem Schlitz (35) in der oberen Platte (34) aufgenommen ist, zwischen oberer und unterer Platte (34, 36), so daß diese eine Patrone (12) durch den Drehtisch (11) in einer aufrechten Stellung getragen wird.

14. Anlage nach Anspruch 13, wobei:
die untere Platte aus einer Federplatte (36) besteht, die elastisch und nachgiebig gegen die Unterseite der oberen Platte (34) vorbelastet ist.

15. Anlage nach Anspruch 10, wobei:
die Reaktorsäule (17) zum Aufnehmen der festen Träger einen geformten Körper aus einem gegenüber den in der Säule (17) behandelten Chemikalien inerten Werkstoff umfaßt, der Körper einen langgestreckten Einlaßteil mit gegenüberliegenden offenen Enden und einen langgestreckten Auslaßteil mit gegenüberliegenden offenen Enden aufweist, die Einlaß- und Auslaßteile am einen ihrer Enden in koaxialer Beziehung zueinander trennbar mit einem Schnappsitz zusammengefügt sind, ein im anderen Ende jedes der Einlaß- und Auslaßteile festgelegter Block aus porösem Werkstoff eine mechanische Barriere gegen ein Herausdringen des festen Stoffs aus der Säule (17) bildet und an jedem der anderen Enden der Einlaß- und Auslaßteile Mittel zur Verbindung der Säule (17) mit einem Fluidliefersystem vorgesehen sind.

Revendications

1. Installation pour procédé chimique automatisé comprenant :
   - une pluralité de cartouches (12) pour emmagasiner un produit chimique, l'une au moins des cartouches (12) servant à emmagasiner un premier produit chimique,
   - un moyen (13) pour choisir l'une quelconque desdites cartouches (12), saisir ladite cartouche (12) choisie et positionner à priori ladite cartouche (12) en un emplacement fixé dans ladite installation,
   - un réservoir (Rn), éloigné dudit emplacement, destiné à contenir un second produit chimique que l'on doit faire réagir avec ledit premier produit chimique se trouvant dans ladite cartouche (12) choisie afin de produire un troisième produit chimique,
   - un moyen (16), au niveau dudit emplacement, destiné à être couplé à ladite cartouche (12) choisie afin d'établir une communication, permettant le passage d'un fluide, avec le contenu de la cartouche,
   - un conduit de fluide (15) relié audit moyen de couplage (16) et audit réservoir (Rn) pour dissoudre et acheminer ledit premier produit chimique de ladite cartouche (12) choisie vers ledit réservoir (Rn) pour le faire réagir avec ledit second produit chimique,
   - un moyen formant vanne (Vn) relié de façon opérationnelle audit conduit de fluide (15) pour réguler l'écoulement dans ledit conduit,
   - lesdites cartouches (12) comportant un moyen (14) formant pompe, actionnable quand ladite cartouche (12) choisie est placée au niveau dudit emplacement pour en modifier le volume interne, afin de provoquer un écoulement bidirectionnel dudit premier produit chimique dans ledit moyen de couplage (16), en le faisant alternativement entrer et sortir du réservoir (Rn) et de la cartouche (12) choisie pour favoriser le mélange et la réaction avec ledit second produit chimique dans ledit réservoir (Rn) pour achever la formation dudit troisième produit chimique,
   - un moyen de commande (18), actionné automatiquement, relié audit moyen formant pompe (14) et audit moyen formant vanne (Vn) pour obtenir une séquence prédéterminée de fonctionnement dudit moyen formant pompe (14) et dudit moyen formant vanne (Vn), afin de réguler la distribution desdits premier et second produits chimiques et le traitement ultérieur dudit troisième produit chimique.

2. Installation selon la revendication 1, dans laquelle une source de pression pneumatique est reliée audit réservoir (Rn) pour mettre son contenu sous pression afin d'aider à faire entrer et sortir l'écoulement dudit premier produit chimique dans ledit réservoir (Rn).

3. Installation selon la revendication 2, dans laquelle ladite installation comprend un appareil de synthèse de protéines et ledit réservoir (Rn) inclut une colonne de réacteur (17) reliée en série avec ledit conduit de fluide (15) pour recevoir ledit premier produit chimique provenant de ladite cartouche choisie (12).

4. Installation selon la revendication 3, dans laquelle ledit moyen de commande actionné automatiquement inclut un ordinateur (18) programmé pour faire fonctionner ledit moyen formant vanne (Vn) et ledit moyen formant pompe (14) suivant ladite séquence prédéterminée.

5. Installation selon l'une quelconque des revendications 1 à 4, dans laquelle ledit moyen formant pompe comprend un piston (14), en-

fermé de façon étanche dans lesdites cartouches (12) en pouvant y coulisser, et un bras alternatif (40), relié audit piston (14) pour le faire aller et venir quand ladite cartouche choisie (12) se trouve au niveau dudit emplacement, afin d'alternativement amener de la matière dans ladite cartouche choisie (12) et expulser ensuite cette matière de ladite cartouche choisie (12).

6. Installation selon la revendication 5, dans laquelle ledit moyen (13) choisissant la cartouche comprend un carrousel rotatif (11) avec des moyens (35, 36) pour y saisir et y supporter lesdites cartouches (12), et ledit moyen de commande (18) est relié pour actionner ledit carrousel (11) suivant une séquence synchronisée avec le fonctionnement dudit moyen formant vanne (Vn) et dudit moyen formant pompe (14).

7. Installation selon la revendication 6, dans laquelle ledit moyen de couplage (16) inclut un moyen (16) déplaçable pour venir en contact, et s'éloigner, de ladite cartouche choisie (12) au niveau dudit emplacement, et ledit moyen de commande (18) est relié pour actionner ledit moyen de couplage (16) suivant une séquence synchronisée avec le fonctionnement dudit carrousel (11), dudit moyen formant pompe (14) et dudit moyen formant vanne (Vn).

8. Installation selon la revendication 7, dans laquelle des capteurs sont placés à proximité dudit carrousel (11), dudit moyen formant pompe (14) et dudit moyen de couplage (16), pour détecter la position et l'état de ceux-ci et renvoyer en réponse un signal audit moyen de commande (18) afin de garantir le fonctionnement dudit carrousel (11), dudit moyen formant pompe (14) et dudit moyen de couplage (16) selon une séquence synchronisée appropriée.

9. Installation selon la revendication 8, dans laquelle ledit carrousel (11) comprend un élément (34) en forme de disque avec une pluralité de fentes (35) dans son bord périphérique, lesdites cartouches (12) étant supportées dans chacune desdites fentes (35).

10. Installation selon la revendication 3, dans laquelle ledit premier produit chimique dans lesdites cartouches (12) contient un mélange d'acide aminé solide, d'hydroxybenzotriazole et d'hexafluorophosphate de benzotriazolyloxytris(diméthylamino)-phosphonium,

ledit second produit chimique dans ledit réservoir (Rn) contenant de la N-méthylmorpholine dans du diméthylformamide en vue d'une dissolution et d'une activation desdits premiers produits chimiques de ladite cartouche choisie (12) afin de former ledit troisième produit chimique, et

un support solide en polystyrène contenu dans ladite colonne de réacteur (17) pour couplage avec ledit troisième produit chimique activé de ladite cartouche choisie (12).

11. Installation selon la revendication 5, dans laquelle lesdites cartouches (12) comportent une collerette (24) plus large diamétralement, formée sur l'extérieur du corps (20) de celles-ci, entre les extrémités (21, 22), pour fixer lesdites cartouches (12) à un appareil de support.

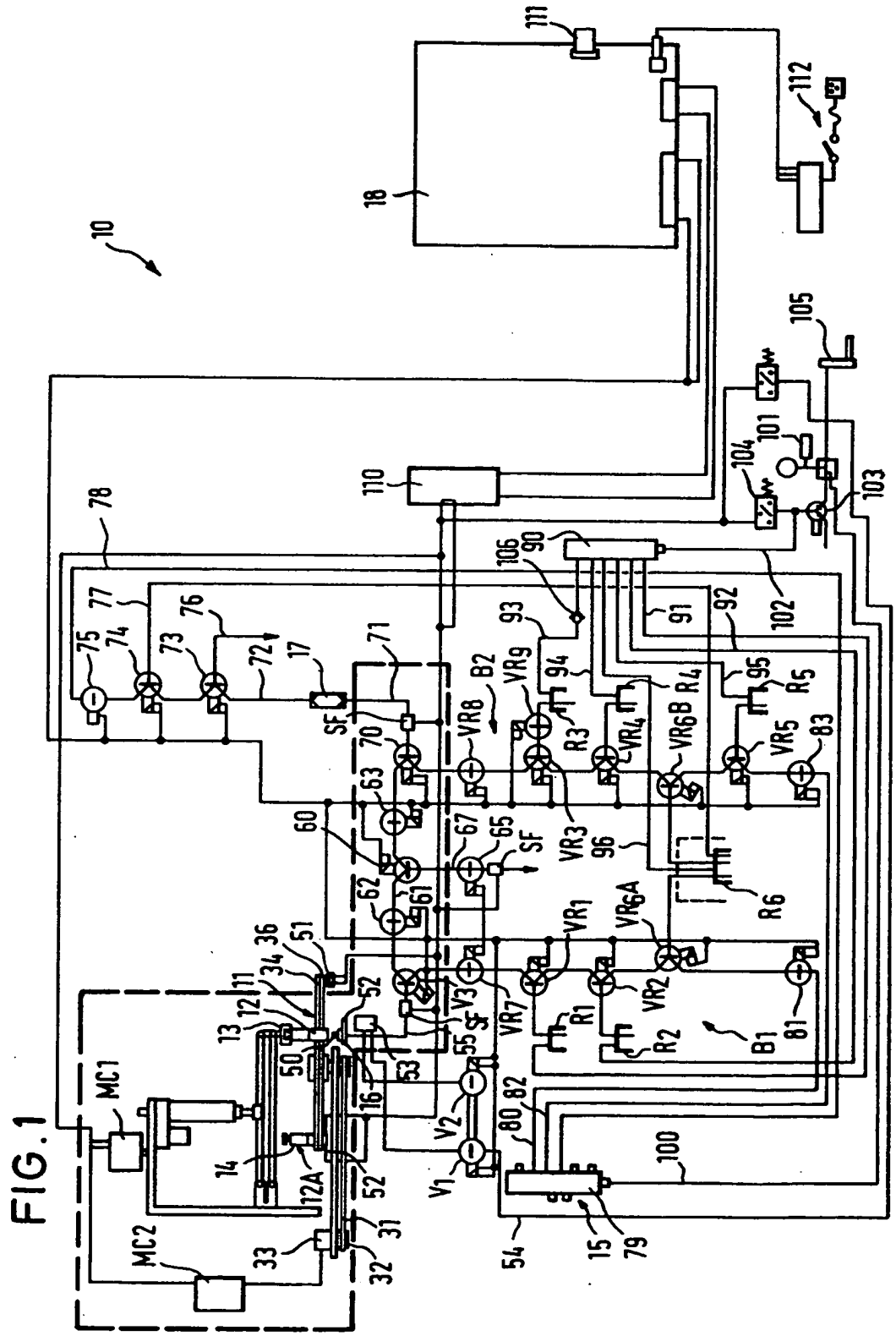
12. Installation selon la revendication 11, dans laquelle un élément poreux (25) est fixé dans ledit corps (20) à proximité de son extrémité inférieure (22) qu'il recouvre pour définir une barrière mécanique empêchant une fuite de produit chimique depuis l'intérieur desdites cartouches (12).

13. Installation selon la revendication 6, dans laquelle ledit carrousel rotatif (11) pour tenir et faire avancer lesdites cartouches (12) ayant une collerette (24) extérieure comporte en outre :
- une plaque supérieure (34) avec un bord périphérique qui a une pluralité de fentes (35) radiales, et
- une plaque inférieure (36) fixée en dessous de la plaque supérieure (34) de façon à pincer entre les plaques supérieure et inférieure (34, 36) la collerette (24) de l'une desdites cartouches (12) logée dans une fente (35) de la plaque supérieure (34), de sorte que ladite cartouche (12) est supportée en position verticale par ledit carrousel (11).

14. Installation selon la revendication 13, dans laquelle ladite plaque inférieure comprend une plaque 36 formant ressort, poussée de façon souple et élastique contre le dessous de ladite plaque supérieure (34).

15. Installation selon la revendication 10, dans laquelle ladite colonne de réacteur (17) destinée à contenir lesdits supports solides comprend un corps moulé en matériau inerte aux produits chimiques qui seront manipulés dans ladite colonne (17), ledit corps comportant une partie d'entrée allongée avec des extrémités

opposées ouvertes et une partie de sortie allongée avec des extrémités opposées ouvertes, lesdites parties d'entrée et de sortie étant accrochées par enclenchement libérable l'une à l'autre au niveau de l'une de leurs extrémités, en étant coaxiales, un bloc de matériau poreux fixé dans l'autre extrémité de chacune des parties d'entrée et de sortie fournit une barrière mécanique contre une fuite de matériau solide provenant de la colonne (17), et un moyen sur chacune des autres extrémités des parties d'entrée et de sortie sert à la connexion de ladite colonne (17) à un dispositif de décharge de fluide.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18
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R5
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R3
R6
R2
R1
77
76
75
74
73
72
17
71
SF
B2
VR9
VR8
VR4
VR6B
VR5
83
70
63
60
67
65
VR3
96
62
61
VR1
VR6A
VR2
B1
81
36
51
34
11
12
13
52
V3
VR7
55
50
53
16
V2
80
82
100
14
12A
V1
54
15
79
MC1
MC2
33
32
31

FIG. 1

FIG. 2

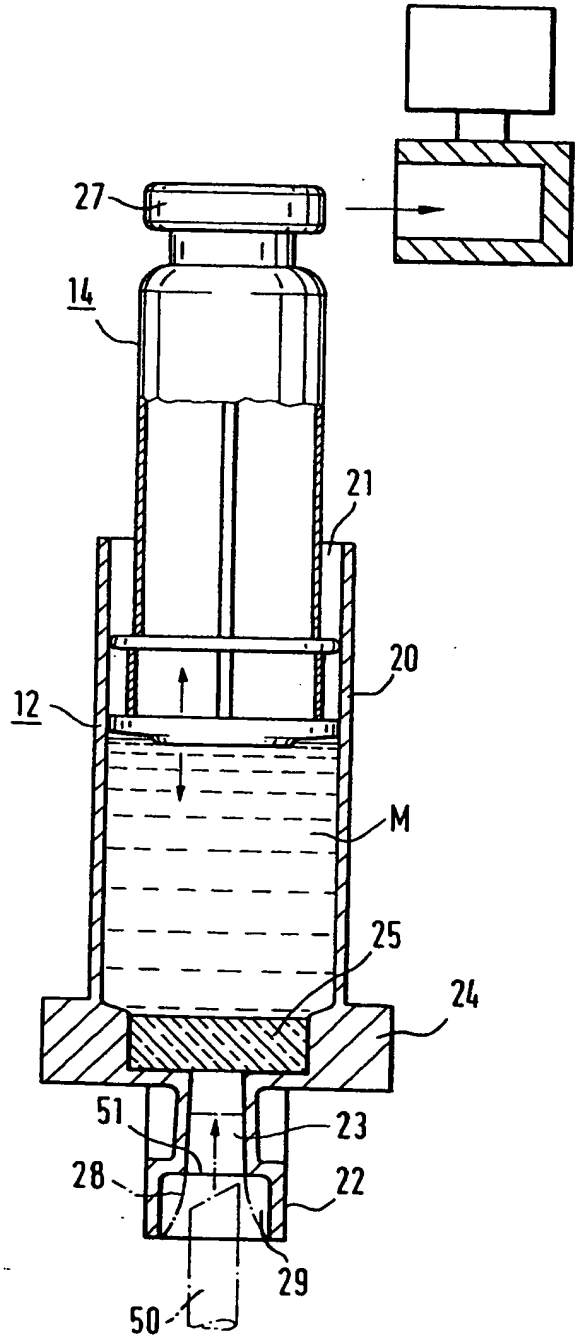

FIG.3

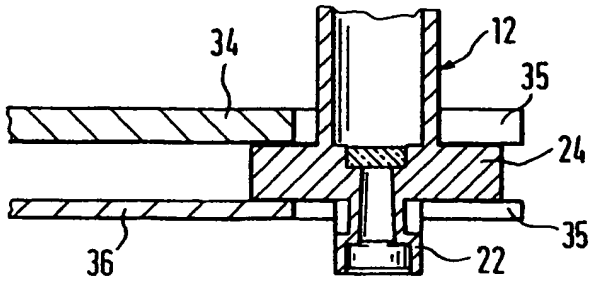

FIG.4

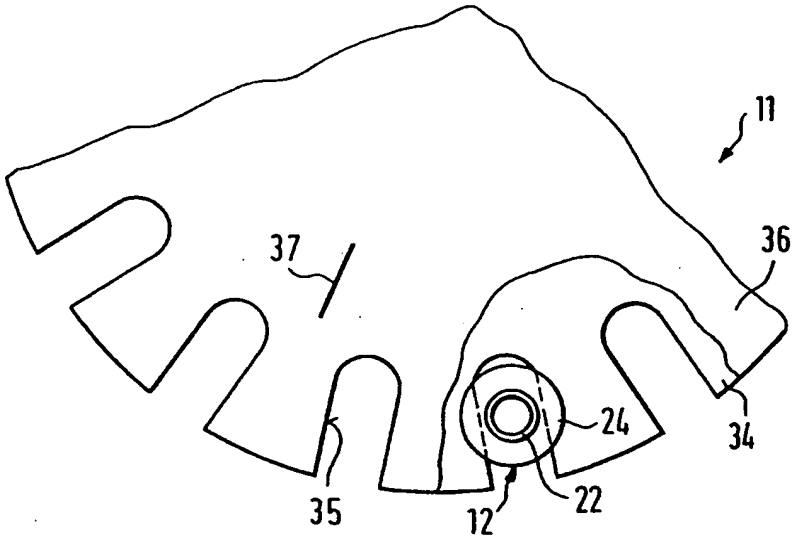

FIG.5

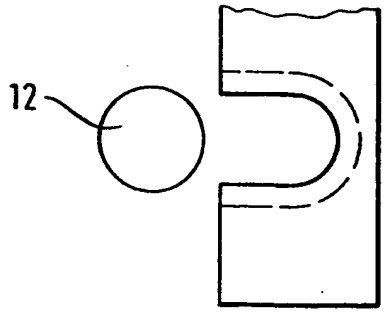

FIG.6